PYRIDOXINE AND SURVIVAL OF TILAPIA (Sarotherodon mossambicus PETERS)

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ABSTRACT

Pyridoxine requirements of tilapia *(Sarotherodon mossambicus* Peters) were studied in two separate experiments using case in-based diets. In Experiment 1, fish on pyridoxine supplemented diet (14.0mg/100g diet) showed no adverse symptoms and remained healthy while fish on a pyridoxine-free diet showed abnormal behaviour with high mortality.

Graded dietary pyridoxine (0.13 to 3.52mg/100g diet) was used in Experiment 2. Lower dietary supplementations of pyridoxine resulted in reduced weight increase, high mortality, high ratio of serum glutamate-oxal-acetate transaminase glutamate-pyruvate transaminase, and reduced blood sugar. The results suggest the dietary requirement of pyridoxine may be between 0.5g and 1.17mg/100g diet; higher supplementations did not appear to confer any further benefits.

INTRODUCTION

The need for pyridoxine in fish has been investigated by some workers. Deficiency symptoms of pyridoxine in fish, appearing 2-9 weeks after pyridoxine deprivation include anorexia, poor growth, hyperirritability, anaemia, nervous disorder, tetany and high mortality (Halver, 1972).

An extensively used clinical parameter is the tissue amino-transferase activities of pyridoxine-deficient animals. Smith *et al* (1974) observed a decrease in erythrocyte glytamic pyruvic transaminase (GPT; Ec. 2.6. 1.2; Alanine amino-transaminase) activity and an elevated liver GPT during pyridoxine deficiency in rainbow trout *(Salmo gairdneri).* Ogino (1965) showed increased liver glumatic exalacetic transminase (GOT, EC 2.61.1; Aspartate amino transaminase) and GPT with increases of dietary pyridoxine intake for carp *(Cyprinus carpio);* similarly with the liver and muscle GOT and GPT activities in turbot *(Scophthalmus maximus)* during dietary pyridoxine treatment (Adron et al, 1978). However, Bell (1968) obtained elevated plasma and serum GOT in poisoned and diseased salmon when compared with healthy salmon.

These studies were designed to investigate the need for pyridoxine in *Sarotherodon massambicus,* the amount needed for maximum growth, and the effects of various dietary supplementations of fish performance and composition.

MATERIALS AND METHODS

Progeny of laboratory-reared broddstock of tilapia, *Sarotherodon mossambicus,* Peters (Cichlidae) were used. In experiment 1 we investigated the need for pyridoxine; fifty fish were randomly distributed between two 10-litre fibre-glass central self-cleaning tanks to give 25 fish per tank. Mean weights of fish at the start of the experiment were 5.2 + 1.4 g and 5.6 + 0.6 g for pyridoxine free diet and pyridoxine supplemented diet, respectively.

In experiment 2, we studied the effects of graded supplementations of pyridoxine in the diet of tilapia; six 50-litre central self-cleaning plastic tanks were randomly stocked at 10-16 fish per tank. The tanks were connected to a close circuit water supply, heated to 26.5oC. The quality of the water was monitored for temperature, oxygen, pH, NO_2 and NH_3 twice weekly throughout the experimental period. Both experiments continued for 12 weeks.

Diet Preparation and Feeding

The dietary ingredients (Table 1), obtained from Sigma Chemical Co. Louis, were prepared in the same way; the only variable component being pyridoxine hydrochloride. For experiment 1, we used a pyridoxine-free diet (PFD) with no pyridoxine added, and pyridoxine supplemented diet (PSD) with 14 mg/100g diet. In experiment 2, supplementations varying from 0.13 to 14.0 mg pyridoxine-HCL/100g diet were prepared. Having carefully weighed out the ingredients, we dissolved the fat-solube vitamins in a portion of the corn oil, and the water-soluble ones in water, before mixing them with the other ingredients. Sufficient water was used to blend the mixture into a stiff dough in a Kenwood mixer, and it was then forced through a 4-5 mm aperture to form long strands. After air-drying for 12 hours at room temperature, they were broken and sieved to produce a diet of 2-3 mm particle sizes and stored at -15oC until used. The diets were fed at 3% of the fish body weight per day, adjusted weekly after weighing. Fish were fed three times a day on week-days, and once daily at week-ends.

For weighing, fish were transferred to a solution of benzocaine (0.5g/3 litres of water) for which the benzocaine was first dissolved in a small amount of acetone. When anaesthetized, surplus water on fish was blotted off with absorbent paper, and they were then weighed on a balance to + 0.01g.

Analysis

Specific growth rates (SGR) were calculated according to Brown (1957). At the termination of experiment 2, blood samples were taken for four fish per treatment, as described by Blaxhall and Daisley (1973), and pooled for glucose analysis using a Beckman Autoglucose Analyser. The pooled sera were also analysed for GOT and GPT using the colorimetric method of Hawk (1965). Moisture, fat, ash and protein contents of fish were determined by the standard methods of Association of Official Analytical Chemists (1970).

RESULTS

Experiment 1

Water temperature in all tanks for the period of both experiments was within the range 25.5 to 27.5oC. Other measures of water quality were always satisfactory.

Feeding remained normal until the third week when some of the fish on the PFD ceased active feeding. Mortalities first occurred 1 week after the start in the PFD (Figure 1) with characteristics behavioural changes of fish. When disturbed they swam rapidly in an erratic manner, and at times they swam on their backs with rapid movement and flexing of the opercula. These convulsive motions continued for 1 or 2 hours with the body in tetany before the fish eventually died, symptoms were observed within 2 weeks of feeding fish PFD, and larger size of fish were the first to die from the deficiency symptoms.

Out of the total dead fish on PFD, over 50% of them died within 12 – 24 hours following treatment with benzocaine for weighing, thus indicating that they were perhaps less resistant to stress. Mortality among fish on the PFD was high, reaching 44% by week 6, whereas none of the fish on the PSD had died at this time.

At the start of week 6, fish on PFD were returned to the PSD to confirm whether there was any correlation between the diet treatment and the symptoms and mortalities observed. Fish regained their appetite and no longerdisplayedthe behaviourral changes. Only one fish died within the week following the return to the PSD.

Experiment 2

Similar behavioural symptoms observed in fish on PFD were noted in fish on the lowest supplementation of dietary pryridoxine HCL, and mortality was highest in this group (Figure 1). The mean body weight of fish and the specific growth rate improved with increase in dietary pyridoxine content up to a maximum (P4) after which further pyridoxine HCL addition had no effecct. An increasing trend in blood sugar value was obtained in the sampled fish, while the sera GOT varied randomly with no correlation to the pyridoxine intake whereas the GPT values increased with higher pyridoxine intake to a maximum (P4) before declining off. The GOT/GPT ratios decreased from 3.4 to 1.2 with increased dietary pyridoxine HCL supplementations Table 3)

DISCUSSION

Behavioural deficiency symptoms observed in experiment 1 become apparent by week 2, and were similar to those reported in the literature in which development varied from 2–9 weeks in various species examined. The performance of the fish in experiment 2 improved as the dietary pyridoxine increased; mortality decreased to zero with the P5 and P6 diets, and there was a larger percentage weight increase from P1 through to P4. The lower figures (and the consequently low (SGR) for P5 and P6 may be due to their higher initial weights; large fish grow more slowely than small fish. Andrew and Murai (1979) reported anaemia in catfish fed higher amounts of pyridoxine, which might influence growth adversely. Anaemia (among other haematological changes) was observed in pyridoxine-deficient salmonids and cyprinids (Halver, 1957; 1972; Ogino, 1965; Smith *et al*, 1974).

The blood sugar of the fish increased with dietary pyridoxine supplementation. A similar observation was reported in rats by Huber *et al* (1964) who suggested that hormonal derangements (adrenaline and glucagon) might be responsible.

A reduction in tissue protein was observed in fish fed the lowest amount of pyridoxine, although the protein in ther diet was the same as that of fish receiving more pyridoxine. Various enzymes of non-oxidative amino-acid metabolism are dependent on pyridoxal–5[1] -phosphate, any any impairment in their activity would thus affect the protein status of the fish. There was no correlation between pyridoxine supplementation and carcass fat deposition in the present study, although fatty or cirrhotic liver have been reported in monkeys deprived of this vitamin (Sauberlich, 1968).

Serum GPT showed an increasing trend with dietary pyridoxine (apart from one aberrant) value with the P5 treatment), while no pattern was seen in the serum GOT, agreeing with observations in other species (Caldwell and McHenry, 1953; Brin *et al*, 1960; Lumeng *et al*, 1978).

The GOT/GPT ratio is commonly used to assess the behaviour of these enzymes in various deficiency states; in rats, a ratio of 1.3: 1 is taken as normal (Hawk, 1965; Bergmeyer, 1974; Lumeng *et al* 1978). In the present study, this ratio was attained with the P3 and P4 treatments; this is believed to be the first time that the parameter has been employed for fish on pyridoxine treatments. Perhaps subsequent findings may define this ratio for fish. However, this ratio was obtained within the treatments where maximum growth and SGR were attained.

TABLE 1 – DIETARY INGREDIENTS USED IN PYRIDOXINE

Major Nutrients (g/100g Diet)

Casein (Vitamin-free)	35.0	Alpha cellulose	21.0
Corn Oil	12.0	Vitamin mix[1]	2.0
Codliver Oil	6.0	Mineral mix2	3.0
Dextrin	10.0	Carboxymethyl	
Alpha Starch (Potato)	10.0	Cellulose (C.M.C)	0.5
		Chromic oxide	0.5

1. The vitamin mix constituent (in mg/100g diet: thiamine HCL 14.0 riboflavin 45.00; nicotinic acid 60.00; calcium pantothenate 95.00; inositol 500.00 ascorbic acid 350.00; choline chloride 780.00; biotin 1.50; folic acid 3.50; para-amino benzoic acid 65.00; cyanocobalamin 0.15; alpha tocopheryl acetate 64.00; menadione 6.00.

2. The mineral mix contained (in g/100g mix): major minerals: calcium orthophosphate 13.6; calcium lactate $5H_20$ 32.7; ferric citrate $5H_20$ 3.0; magnesium sulphate $7H_20$ 13.2; dipotassium hydrogen orthophosphate 24.0; di-sodium orthophosphate 8.7; sodium chloride 4.4; Trace minerals: alluminium chloride (anhydrous) 0.008; potassium iodide 0.013; zinc sulphate $7H_20$ 0.15; manganese sulphate H_20 0.08; cobalt chloride $6H_20$ 0.10.

TABLE 2 – PERFORMANCE OF S. MOSSAMBICUS ON DIFFERENT AMOUNTS OF DIETARY PYRIDOXINE (EXPERIMENT 2)

Pyridoxine Supplementation (mg/100g Diet)	*0.13*	*0.25*	*0.52*	*1.17*	*3.52*	*14.00*
Treatment Code	P1	P2	P3	P4	P5	P6
Initial Number of fish	16	14	15	15	13	10
Final Number of fish	11	12	14	14	13	10
Mortality (%)	31.3	14.3	6.6	6.6	0	0
Mean Initial	11.8	10.4	11.2	11.3	12.9	17.1
Weight (g) ± S.D.	±5.6	±4.2	±7.0	±6.7	±5.8	±4.4
Mean Final	18.5	19.8	22.8	23.9	21.6	25.3
Weight (g) ± S. D.	±6.9	±4.3	±9.9	±10.3	±5.5	±7.8
% Increase In	55.6a	89.0a b	104.0b	112.1b	67.7a	48.3a
Mean Weight ± S.D.*	±17.9	±12.0	±18.8	±21.1	±12.5	±15.6
Mean S.G.R. ± S.D.*	b.c 0.84±0.5	b.c 0.84±0.3	c 1.00±0.2	c 0.95±0.4	ab 0.67±0.2	a 0.51±0.2

**Figures with the same superscript are not significantly different (Duncan's Multiple Range test, P 0.05)*

TABLE 3 – ANALYSIS OF S. MOSSAMBICUS FOLLOWING VARYING DIETARY PYRIDOXINE TREATMENT

Pyridoxine Supplementation (mg/100g Diet)	*0.13*	*0.25*	*0.52*	*01.17*	*3.52*	*14.00*
Treatment Code	P1	P2	P3	P4	P5	P6
Number of fish analysed	4	4	4	4	4	4
Moisuture % ± S.D.	75.5±1.5	76.8±1.5	70.71±0.2	74.2±1.3	77.1±0.1	72.8±5.0
Fat % ± S.D.	7.7±2.5	11.3±4.3	8.8±1.7	10.2±1.3	8.4±1.3	11.7±2.2
Dry Tissue Protein % S.D.	43.2±7.5a	51.9±4.2b	57.6±5.1b	50.8±3.5b	53.1±1.5b	53.9±6.2b*
Blood Glucose (mg/100ml pooled)	31	41	65	81	188	159
Serumgot (Units/ml)	257	205	185	242	232	216
Serum GPT (Units/ml) pooled	75	90	147	180	117	175
GOT/GPT Ratio	3.4	2.3	1.3	1.3	2.0	1.2

**Figures with the same superscript are not significantly different (Duncan's Multiple Range Test P = 0.05).*

Comparison of the various analysis performed indicates the possibility of using the blood sugar value as an inexpensive and simple method for diagnosis of pyridoxine deficiency in fish, but since it is not specific for this deficiency, it would need to be assessed alongside observation of behavioural symptoms which show up rather early in this species.

CONCLUSION

Based on the high mortality observed in this fish, pyridoxine – HCL is an essential dietary requirement for *S. mossambicus* and between 0.59–1.17mg pyridoxine–HCL/100g of diet enhanced optimum fish growth with subsequent reduction in mortality.

ACKNOWLEDGEMENT

This study was carried out at the Fish Culture Unit of the University of Aston in Birmingham (Great Britain), and grant was provided by the University of Port-Harcourt (Nigeria).

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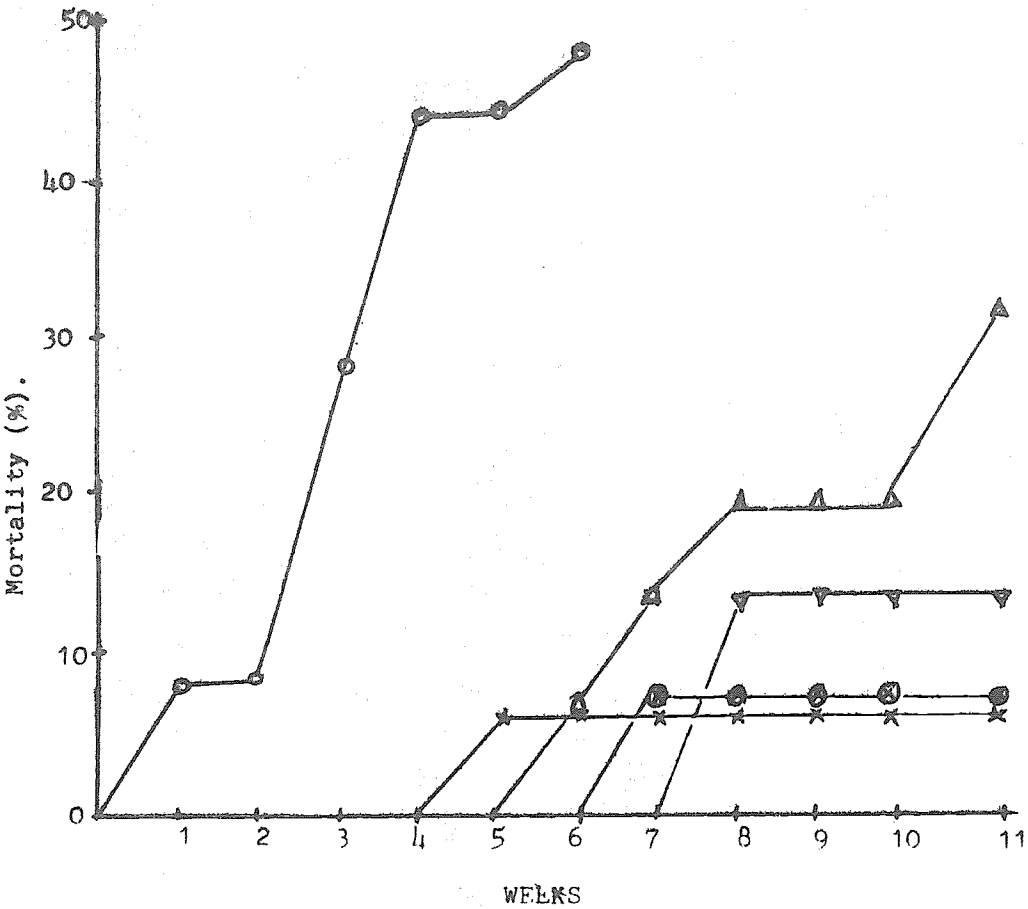

Fig.1: Weekly mortality (%) in Tilapia fed diets containing varying amounts of pyridoxine.

O = O = Γ = F (experiment 1): Δ-Δ = P1; ∇-∇ = P2; ⊗-⊗ = P3;

x = x = P4, (experiment 2).